Field Portable Concentration and Extraction of Pathogen Analytes from Large Volume Blood Samples

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Claim

Levels of bacteremia associated with common bacterial pathogens in adults and children may be less than 1 CFU per ml (3,6). The use of a low volume of blood for a blood culture is known to reduce the detection of bacteremia in adults (5). In an effort to understand how bacterial concentration could contribute clinically significant capabilities to PCR detection systems, blood culture techniques provide relevant information. Tenney et al have shown that 7 ml blood samples resulted in 29 % more positive cultures than found with 2 ml samples (7). A 15 ml culture input sample provided a 25 % greater positive blood culture yield than 5 ml inputs in another study by Hall et al (1). Still others have found improvements for positive blood culture yield when moving from 10 to 20 and 30 ml blood sample inputs (2,4).

1. Hall, M. M., D. M. Ilstrup, J. A. II. Washington. Effect of volume of blood cultured on detection of bacteremia. J. Clin. Microbiol. 3:643-645.

2. Ilstrup, D. M., J. A. II. Washington. The importance of volume of blood cultured in the detection of bacteremia and fungemia. Diagn. Microbiol. Infect. Dis. 1:107-110.

3. Kellogg, J.A., J. P. Manzella, and D. A. Bankert. 2000. Frequency of low level bacteremia in children from birth to fifteen years of age. J. Clin. Microbiol. 38:2181-2185.

4. Li, J., J.J. Plorde, and L. G. Carlson. 1994. Effects of volume and periodicity in blood cultures. J. Clin. Microbiol. 32:2829-2831.

5. Mermel, L. A., and D. G. Maki. Detection of bacteremia in adults: consequences of culturing an inadequate volume of blood. 1993. Ann. Intern. Med. 119:270-272.

6. Reimer, L.G., M. L. Wilson, M. P. Weinstein. 1997. Update on detection of bacteremia and fungemia. Clin. Microbiol. Rev. 10:444-465.

7. Tenney, J. H., L. R. Reller, S. Mirrett, W-L. L. Wang, M. P. Weinstein. 1982. Controlled evaluation of the volume of blood cultured in detection of bacteremia and fungemia. J. Clin. Micorbiol. 15:558-561

Collaborations and Coordination

CDC Bioterrorism RRAT Lab

USAMRIID

MIT LL

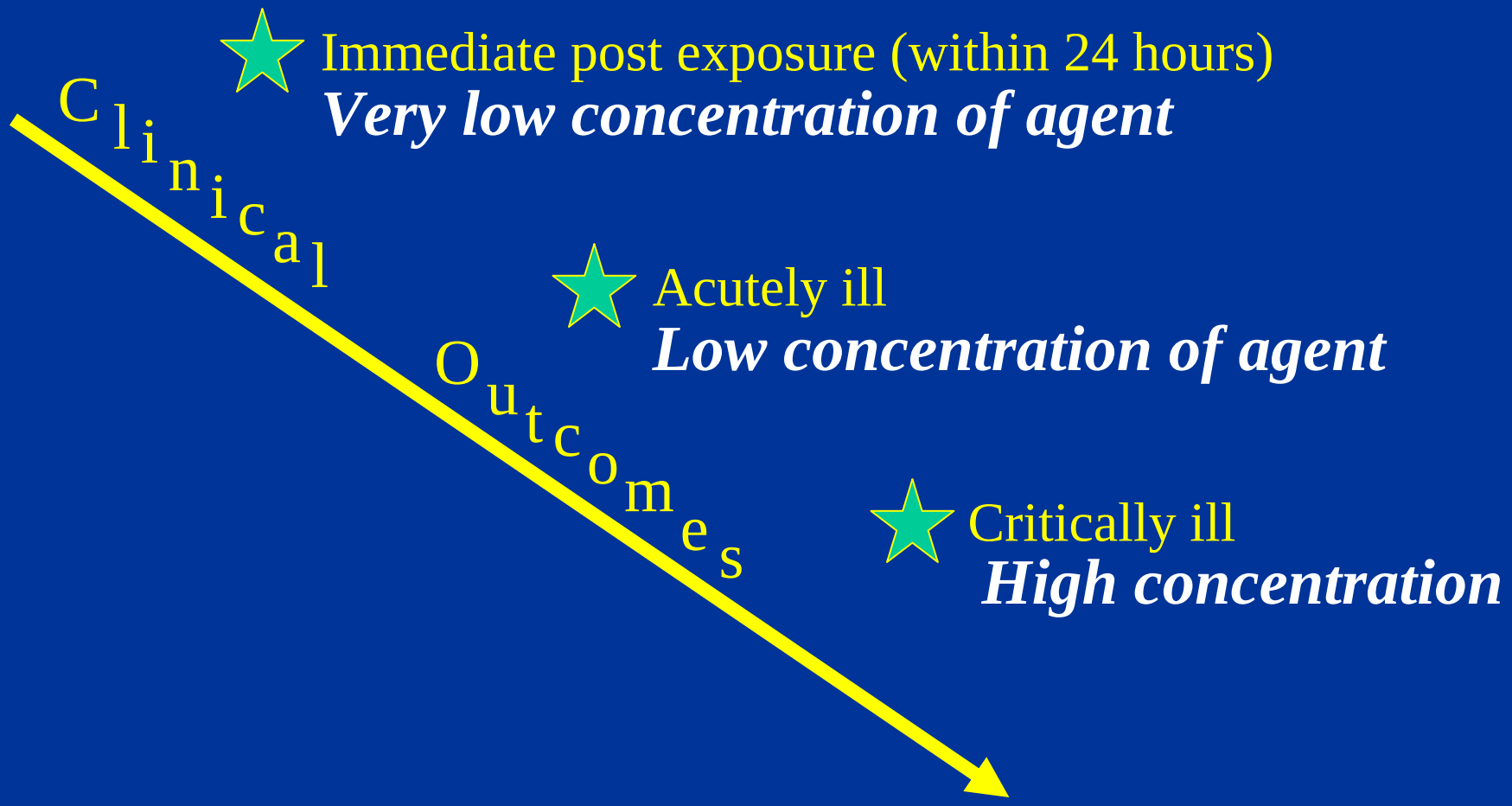
AFIP

UT

Roche Applied Science, Penzburg, Germany

Path Centre, Perth, Australia

Clinical Testing Scenarios



Benefits of a Rapid and Sensitive Protocol for PCR Detection of Bacteria in Blood

B. anthracis **– Possible Early ID of Mediastinitis**

Y. pestis **– Early ID of Septicemic Bubonic Patients**

F. tularensis **– Slight Aerosol Danger from Culture**

Brucella **spp. – Blood Culture Can Take 4 Weeks**

Burkholderia **– Rapid Onset of Sepsis Within 24-48 h**

U.N. Report: Efforts to Halt al-Qaida Failing Terror Group Moving Toward Chemical, Biological Weapons

By EDITH M. LEDERER, AP

UNITED NATIONS (Nov. 15) - The al-Qaida terror network is determined to use chemical and biological weapons and is restrained only by the technical difficulties of doing so, a U.N. expert panel said in a confidential report.

Date: 14 Nov 2003

From:ProMED-mail <promed@promedmail.org>

Source: News 24 website / Xinhua [edited]

Food laced with rat poison killed one and sent 24 to the hospital during a wedding party in east China's Jiangsu province, state media said on Friday.

The mass poisoning happened on Thursday in Sihong county, as several guests started feeling sick shortly after the wedding banquet had started, the Xinhua news agency reported.

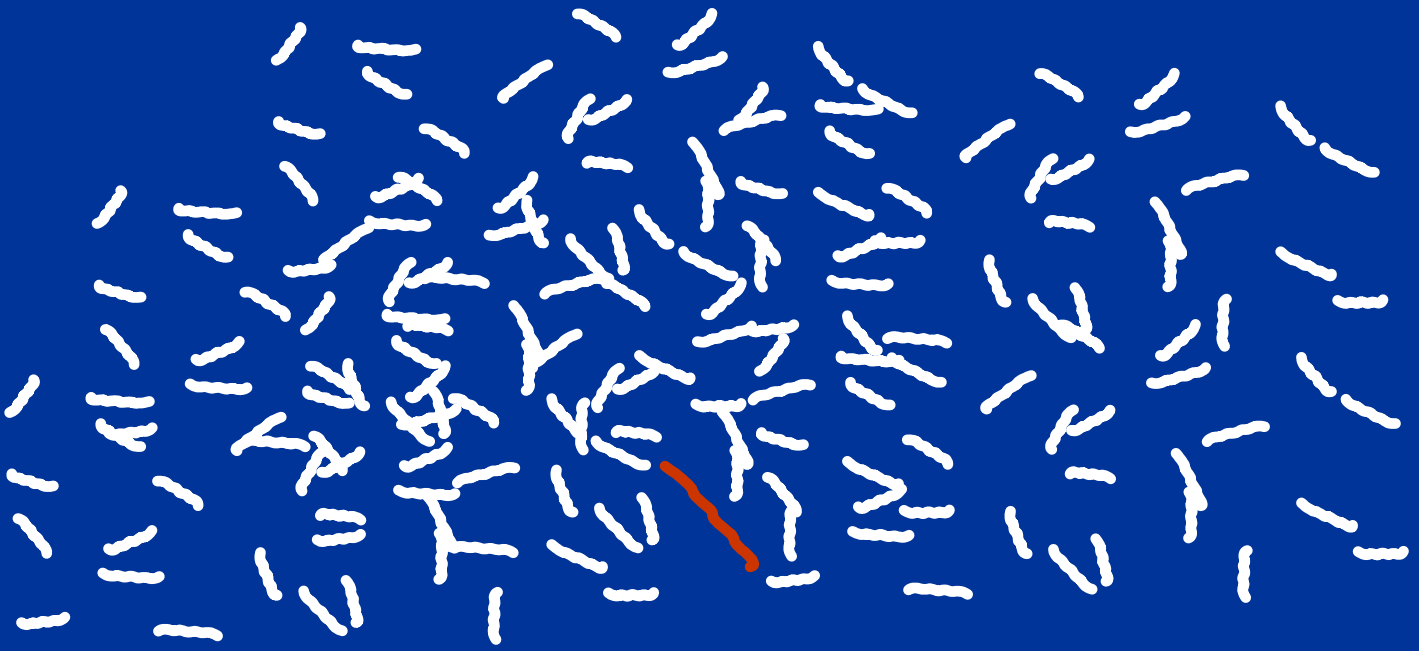
Typical Clinical Specimen Collection Time Line

Time Period	***Bacillus anthracis***	***Yersinia pestis***
0 – 24 h	nasal swab	nasal swab
 72 h	serum	serum sputum

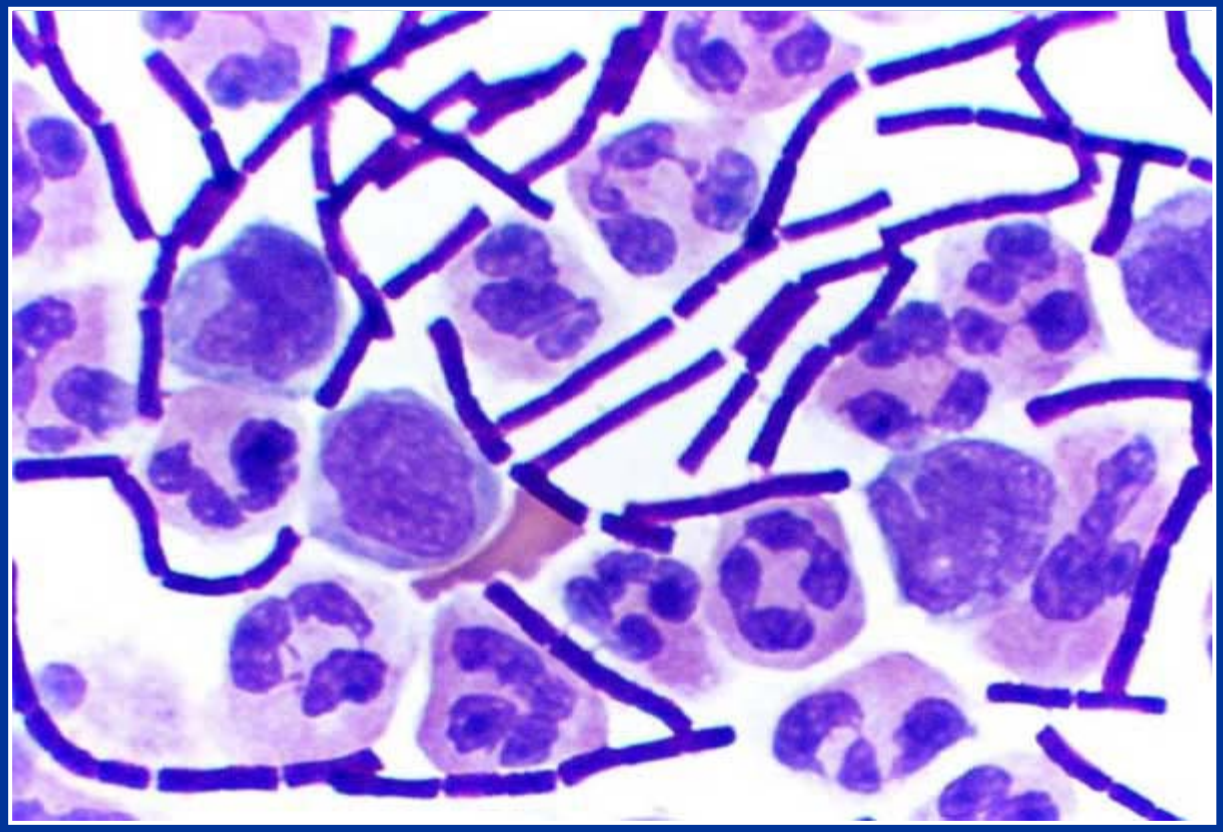
Performance Specifications Objectives

- Field Portable
- Closed System Architecture
- Minimal End User Input / Fully Automated
- RNA and DNA from Bacteria, Virus, and Host Response Using 1 – 8 ml of Whole Blood
- Protection of Host RNA as well as Analyte RNA, DNA and Protein
- Minimize PCR Inhibitory Host DNA and Blood Matrix Biomass
- Rapid Processing Time (analyte capture and elution within 15 minutes / total nucleic acid extraction within 45 minutes)
- Plug and Play Compatibility with All Nucleic Acid, Biosensor, Mass Spec, or Culture Based Detection Systems, and Automated Host Response Array Technologies

Challenge for Rare Analyte PCR Detection in Clinical Samples



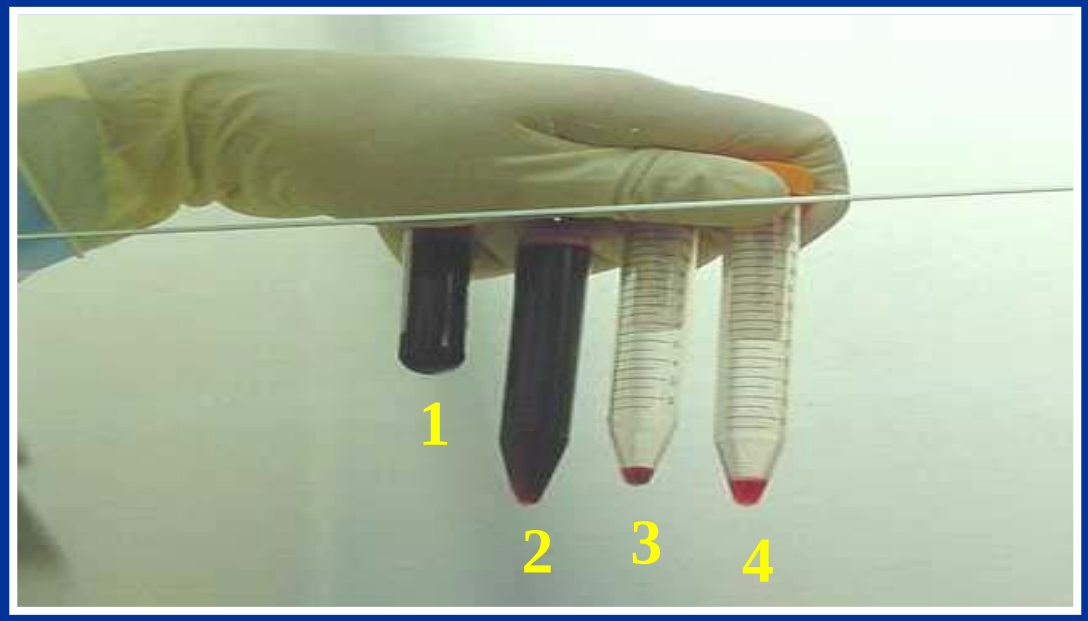
B. anthracis *Gram stain*



Light Cycler Crossing Points for *Burkholderia pseudomallei*

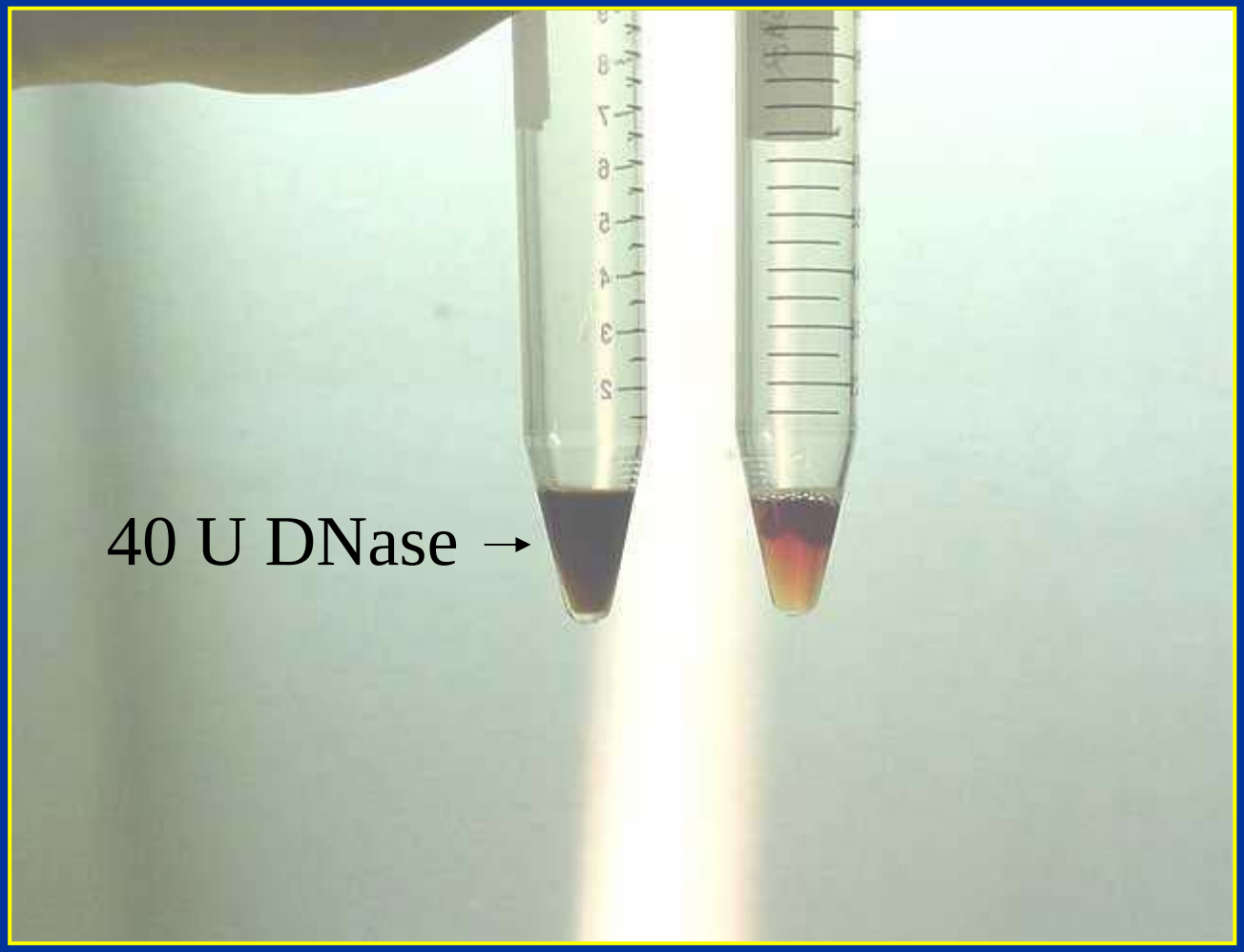
Sample Number	CFU / 6 ml Whole Blood	Crossing Point
1	0.1	0.00
2	0.1	0.00
3	5.0	34.10
4	5.0	37.77
5	10.0	36.19
6	10.0	37.27
7	20.0	38.69
8	20.0	34.96
9	40.0	37.00
10	40.0	34.34

Laboratory of Dr. Tim Inglis and PCR Reagents from USAMRIID

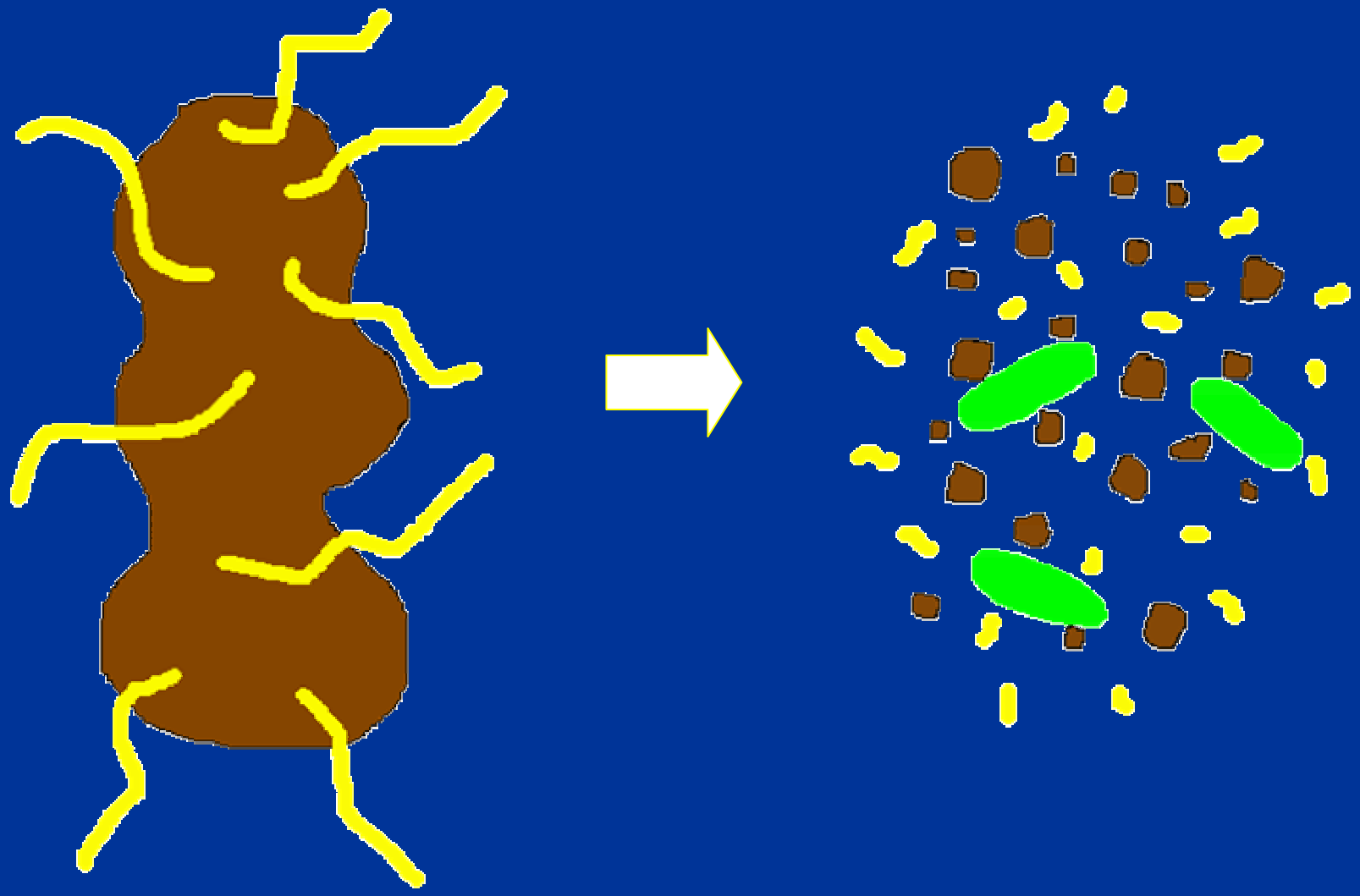


Prep Method: 1) spiked blood, 2) diluted blood, 3) pellet, 4) enzyme treated pellet.

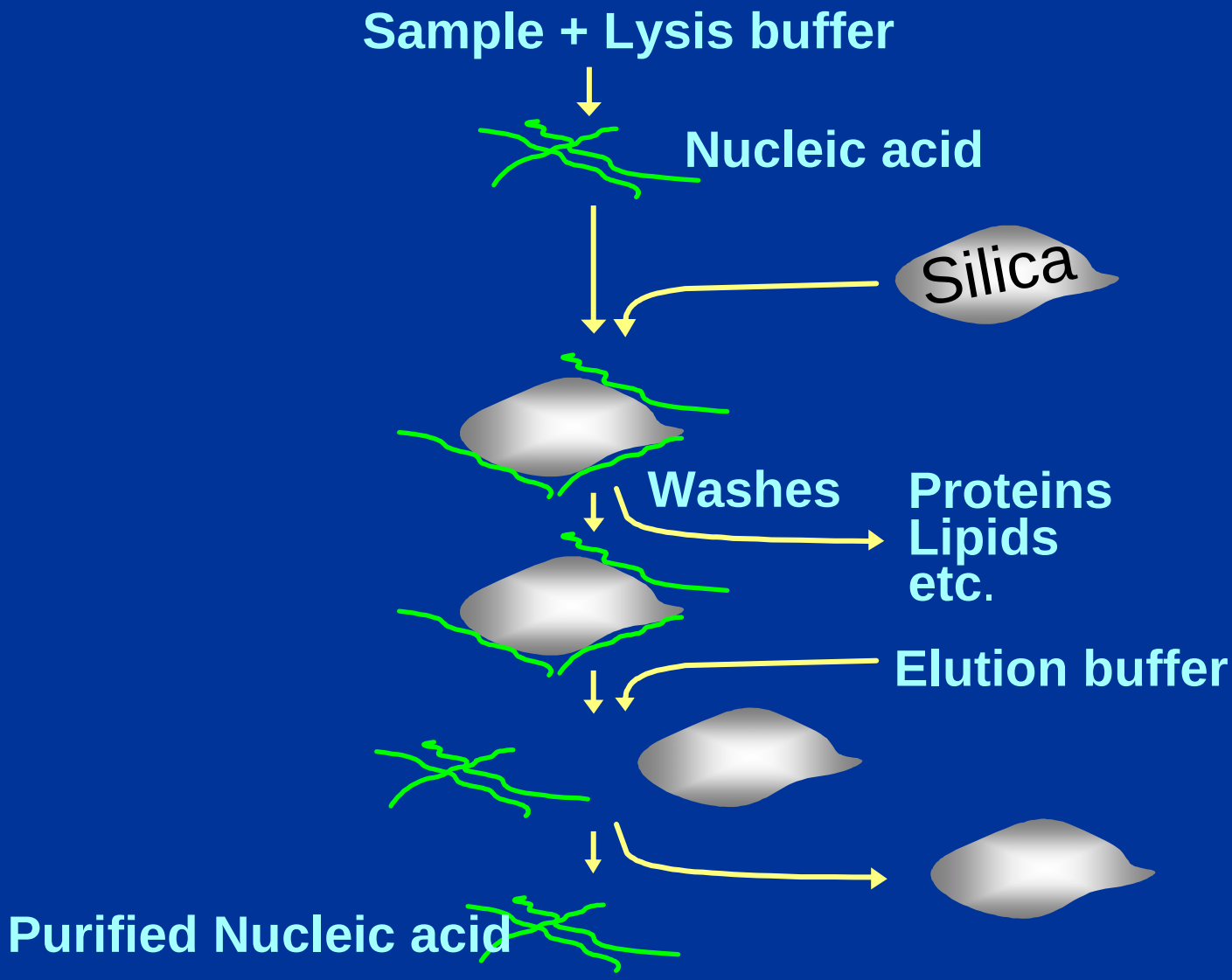
DNase Induced Protein Solubility



DNase Induced Protein Solubility



Sample Lysis and Nucleic Acid Extraction



Whole Blood Seeded with 45 - 75 CFU *Brucella suis* / 6ml

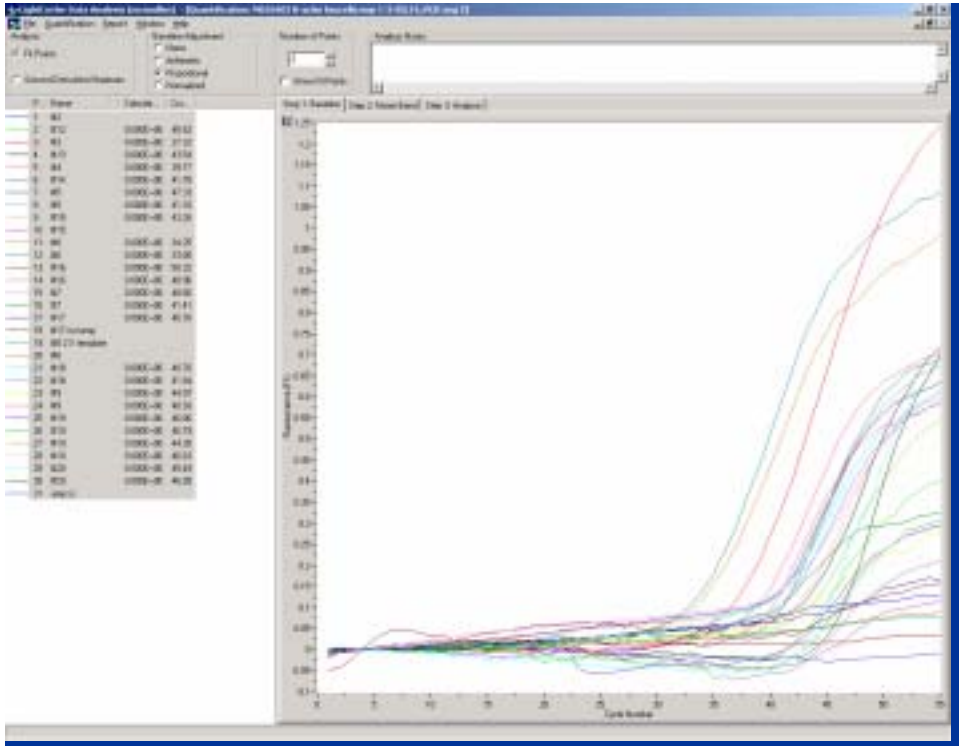
Light Cycler Crossing Points

Spiked Conc. (cfu/6ml)	Sample I.D.*	Oligo Set BRU 1	Oligo Set BRU 2	Oligo Set BRU 3
45	1	38.69		
	2	39.09	42.41	
	3		42.26	
	4			38.12
	5	39.66	37.8	39.56
	6	38.66	39.71	35.67
60	7	37.97	37.36	
	8	41.77	38.36	
	9	39.06	38.06	38.91
	10	42.31	38.85	
	11	39.63	38.91	
	12	39.31	36.32	35.67
75	13	41.28	38.21	
	14	44.72	36.84	
	15	38.57	37.41	38.61
	16	39.03	37.82	38.06

Crossing Points for *B. suis* Samples Processed with and with out Human DNA Removal (HDR)

CFU / 6 ml Blood	Sample Number	No HDR Treatment	With HDR Treatment
20	1	0.00	0.00
20	2	0.00	0.00
50	3	42.11	36.68
50	4	0.00	0.00
100	5	40.19	36.88
100	6	0.00	39.24
200	7	44.17	38.58
200	8	0.00	36.91
400	9	40.45	34.48
400	10	43.29	35.67

Only 50 % of the untreated spiked samples produced positive signals. Ninety percent of the HDR treated spiked samples produced positive signals. The HDR treated samples produced earlier crossing points and 50 to 100 % higher fluorescence signals.

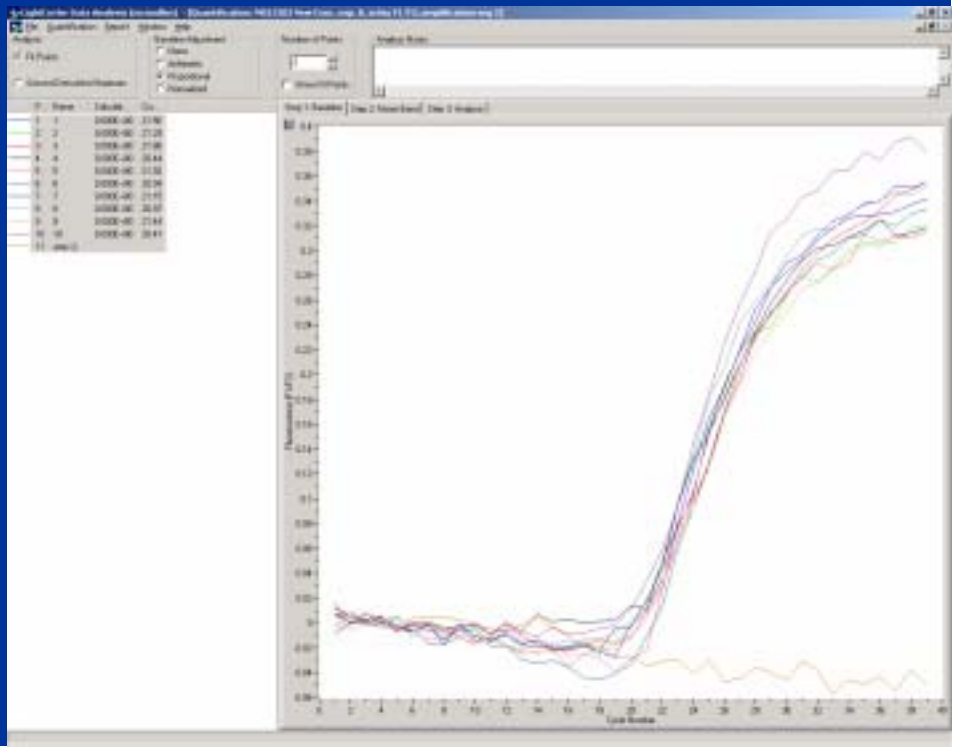


Left. Human DNA Amplification with CDC's Beta actin oligo set using CBD BB004.0 Protocol.

The crossing points ranged from 34.25 to 47.33 with a standard. deviation of 4.6 and a C.V. of 11.15. Note the broad range of total florescence values.

Right. Human DNA Amplification with CDC's Beta actin oligo set using CBD BB005.0 Protocol.

The crossing points ranged from 20.41 to 21.92 with a standard deviation of .0.52 and a C.V. of 2.42. Note the narrow range of total florescence values.



Whole Blood Seeded with 10-80 CFU *Yersinia pestis* / 6ml

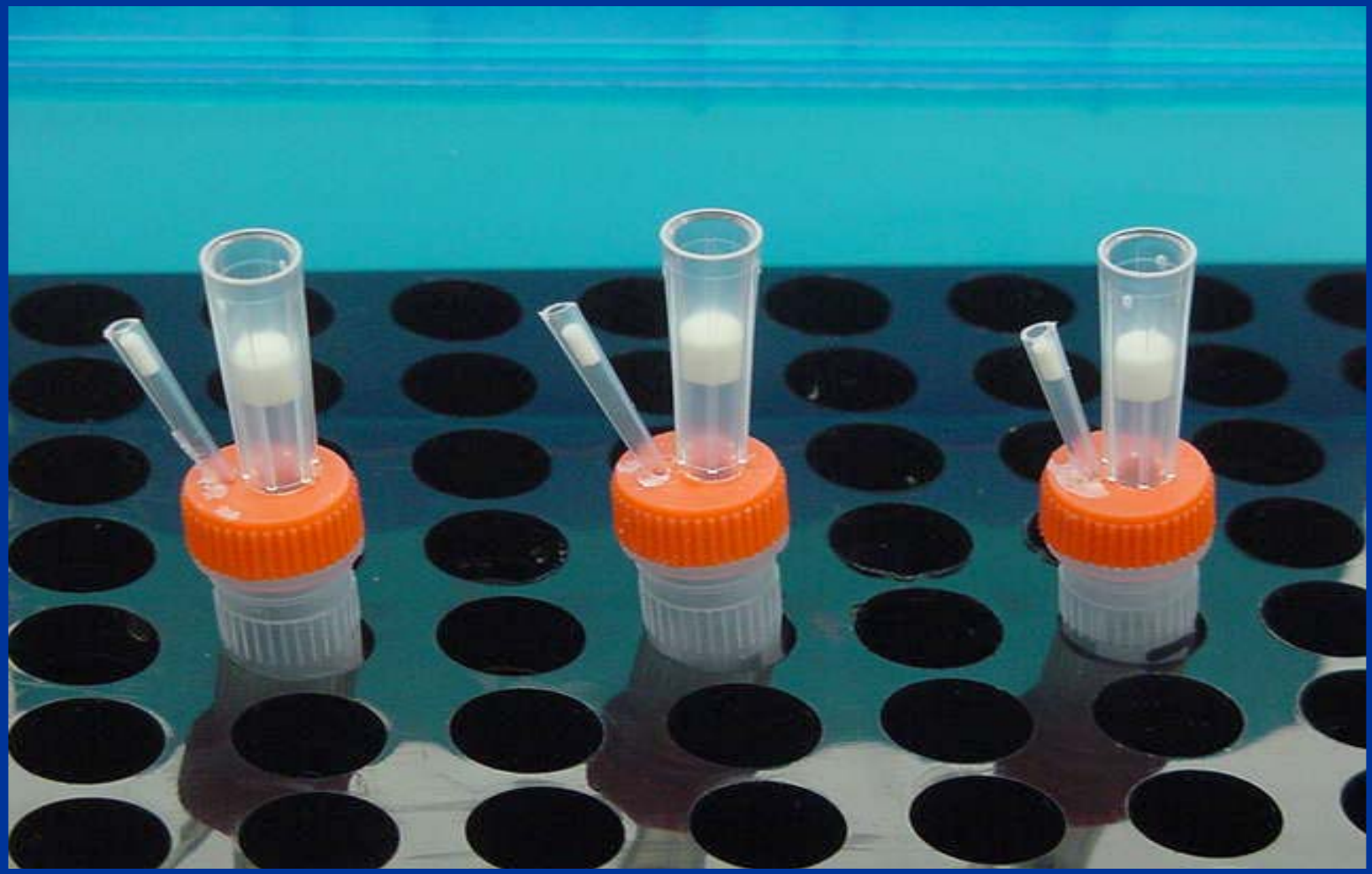
Light Cycler Crossing Points

Spiked Conc. cfu/6ml	Sample I.D.	YP2 Event A			YP2 Event B			YP9 Event A			YP9 Event B		
		AVG.	S.D.	C.V.	AVG.	S.D.	C.V.	AVG.	S.D.	C.V.	AVG.	S.D.	C.V.
1	1												
	2												
10	3							42.90	0.82	1.91	41.90	0.35	0.84
	4							41.99	0.87	2.07	42.26	0.49	1.16
20	5	37.98	1.78	4.69	38.09	0.62	1.63	41.19			42.59	0.22	0.52
	6	37.04	0.30	0.81	39.01	1.93	4.95	42.18	0.79	1.87	42.89	1.33	3.10
40	7	36.91	0.35	0.10	36.94	0.13	0.35	41.03	0.78	1.90	41.80	0.59	1.41
	8	38.51	0.08	0.21	37.33	0.37	0.99	42.39	0.42	0.99	40.94	0.31	0.76
80	9	38.01	0.05	0.13	36.63	0.27	0.74	40.25	0.11	0.27	39.91	0.21	0.53
	10	36.61	0.40	1.10	36.87	0.14	0.38	40.18	0.40	1.00	39.39	0.48	1.22

Spiked Conc. cfu/6ml	Sample I.D.	YP12 Event A			YP12 Event B			YP16 Event A			YP16 Event B		
		AVG.	S.D.	C.V.	AVG.	S.D.	C.V.	AVG.	S.D.	C.V.	AVG.	S.D.	C.V.
1	1												
	2												
10	3	35.82			36.48	0.23	0.63	37.30	0.63	1.69	36.10	0.40	1.11
	4	36.00	0.22	0.61	35.82	0.06	0.17	35.23	0.24	0.68	37.45	0.16	0.46
20	5	35.97	0.21	0.58	36.43	0.45	1.24	36.35	1.30	3.58	36.17	0.64	1.77
	6	36.15	0.31	0.86	36.47	0.14	0.38	35.17	0.36	1.02	34.10	0.20	0.59
40	7	35.46	0.27	0.76	35.88	0.09	0.25	33.11	0.26	0.79	33.97	0.23	0.68
	8	36.42	0.15	0.41	35.68	0.06	0.17	33.74	0.04	0.12	35.22	0.51	1.45
80	9	35.72	0.12	0.34	35.32	0.32	0.91	33.19	0.02	0.06	33.37	0.33	0.99
	10	35.15	0.31	0.88	34.78	0.10	0.29	33.58	0.41	1.22	32.60	0.25	0.77

Dilution Tubes for Vegetative B. anthracis:

Used to Achieve S.D. < 5 % on Plate Counts



Whole Blood Seeded with ≤ 10 CFU *B. anthracis* / 6ml

Sample #	CDC pX02 Oligo Set Avg. Noise Band Crossing Point	CDC pX02 Oligo Set Standard Deviation	CDC Genomic Oligo Set Avg. Noise Band Crossing Point	CDC Genomic Oligo Set Standard Deviation
1				
2				
3	37.86	0.93	40.39	
4	39.07		40.00	
5				
6				
7			40.77	
8			39.86	
9	38.42	0.54	38.53	0.44
10	36.90	0.27	37.75	0.11
11				
12			39.10	
13			38.61	
14				
15				
16	37.79	0.80	38.39	
17	38.27	0.37	38.68	1.20
18	37.52		39.60	0.63
19	36.19	0.36	37.47	
20	37.56	0.73	38.80	0.10
21	36.71	0.44	38.74	1.02
22	37.09		38.47	
23	38.07	0.80	39.06	
24	37.36		38.37	0.55
25			40.24	
26	38.16	0.73	38.34	0.31
27	37.15	0.30	38.28	0.54
28	39.44	0.29	38.31	0.29
29	37.42	0.33	37.69	0.29

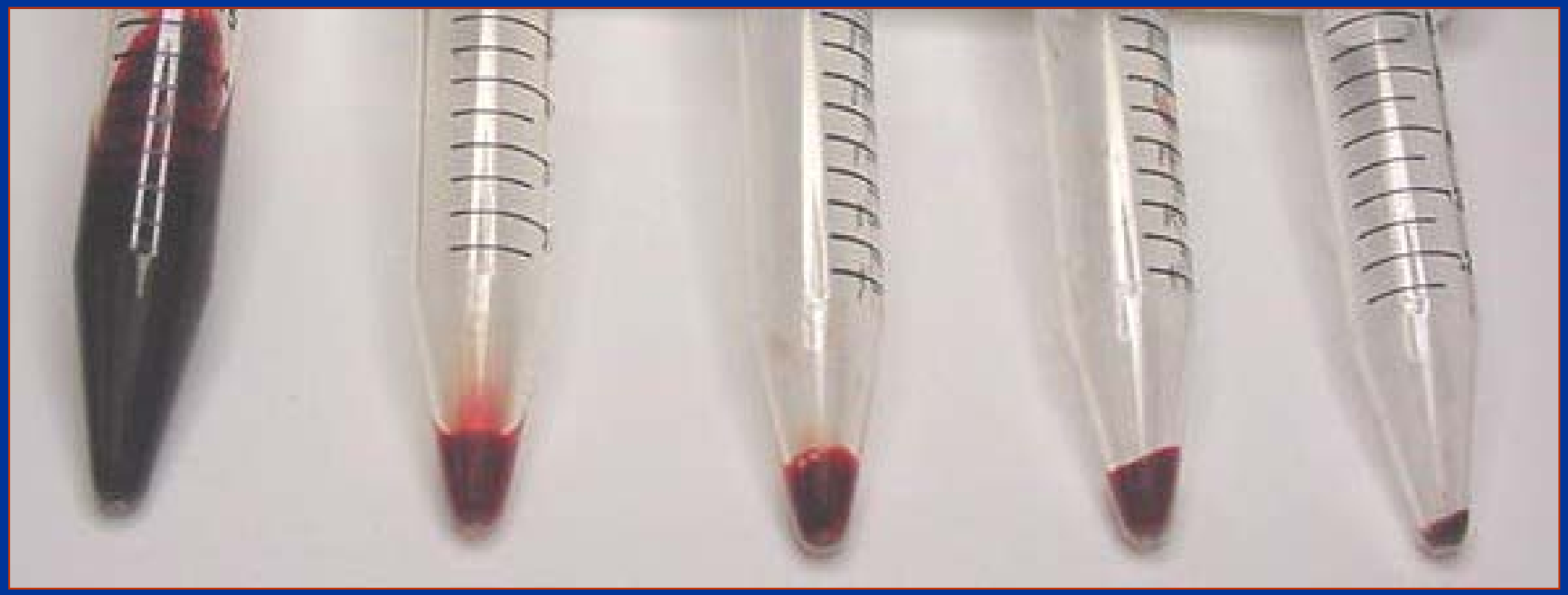
PES Filter Mechanism

Concentration of *B. anthracis* via Filtration and Centrifugation Followed by PCR Analysis

Amount *B. anthracis* Seeded (cfu/6ml)	Centrifugation: Noise Band Crossing Points			Centrifugation: Mean	Centrifugation: Std. Dev.	Filtration: Noise Band Crossing Points			Filtration: Mean	Filtration: Std. Dev.
≤ 0.01										
≤ 0.01										
≤ 10.0						40.33	39.89		40.11	
≤ 10.0							37.79		37.79	
≤ 10.0						40.36		37.69	39.03	
≤ 10.0	41.93		40.31	41.12						
≤ 10.0			40.47	40.47		37.90	37.70	37.79	37.80	0.10
≤ 10.0	38.11		40.36	39.24		36.45	36.09	36.81	36.45	0.36
≤ 50.0	37.56	36.49	37.91	37.32	0.81	35.15	34.91	34.98	35.01	0.82
≤ 50.0	36.45	38.15	38.49	37.70	1.09	35.24	34.18	34.68	34.70	0.53

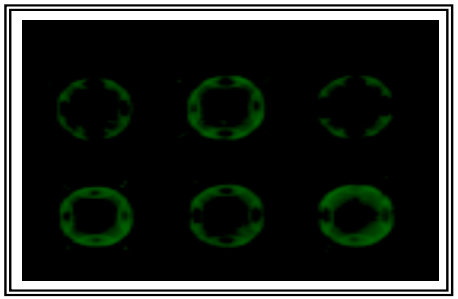
Filtration Method May Be Automated

Six ml Whole Blood With and Without Detergent and Enzyme Treatment Followed by Centrifugation @ 5,500 RCF



Untreated Blood	1 % Saponin	1 % Triton X – 100	Exp. Detergent	Exp. Detergent + Enzymes

Successful CRP dose response for reagent processed whole blood



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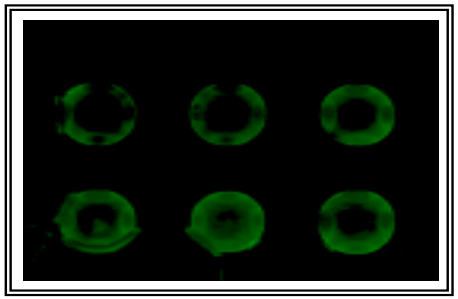
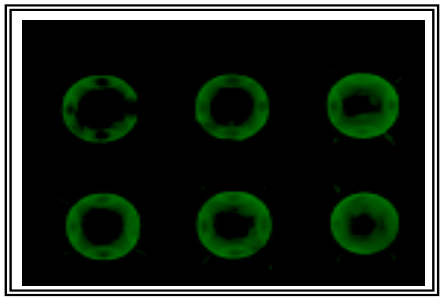
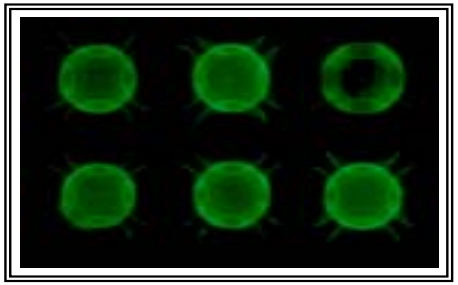


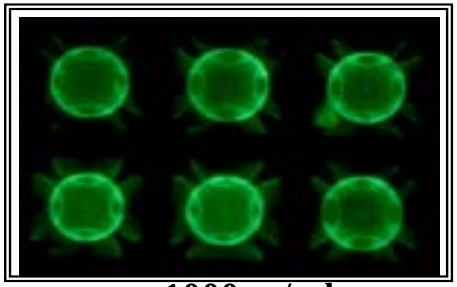
1ng/ml



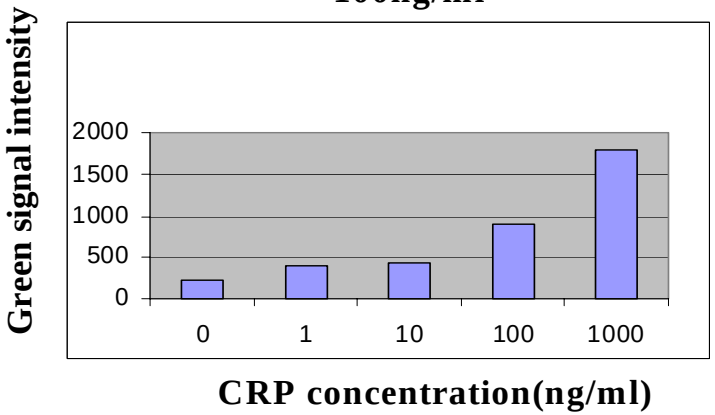
10ng/ml



100ng/ml



1000ng/ml

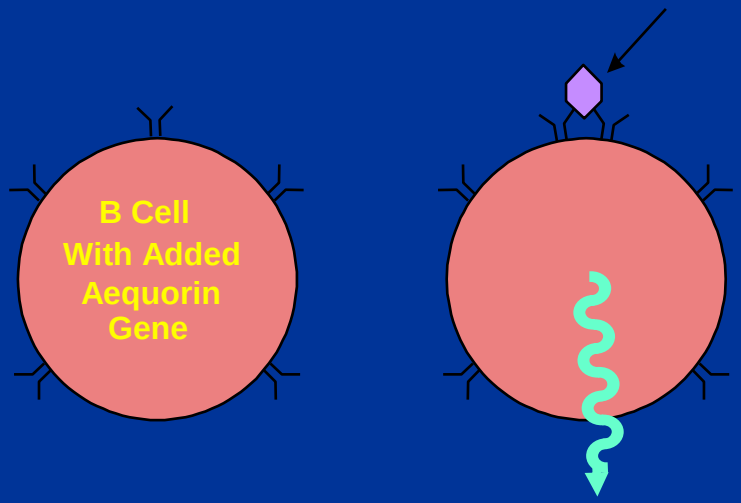


Data acquired at the University of Texas at Austin by the McDevitt laboratory on 6-30-03; Sample provided by Matt Ewert, USF

CANARY Sensor Concept
MIT Lincoln Laboratories

- Responds in seconds
- High sensitivity
- B-cell specificity is engineered with recombinant antibodies
- Aequorin amplifies signal and provides simple interface

(1) Pathogens cross link membrane-bound recombinant antibodies

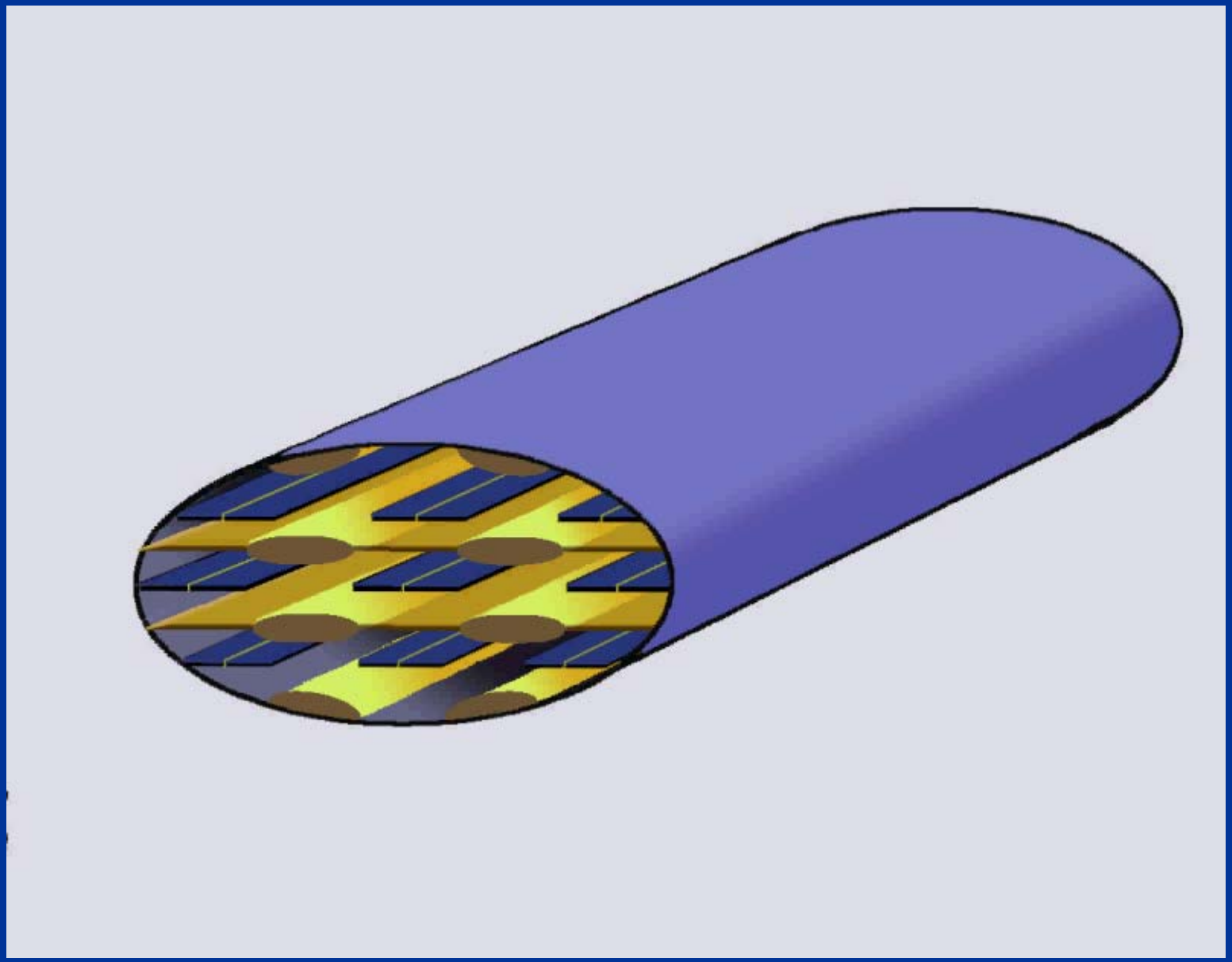


(2) Biochemical signal amplification releases Ca^{2+}

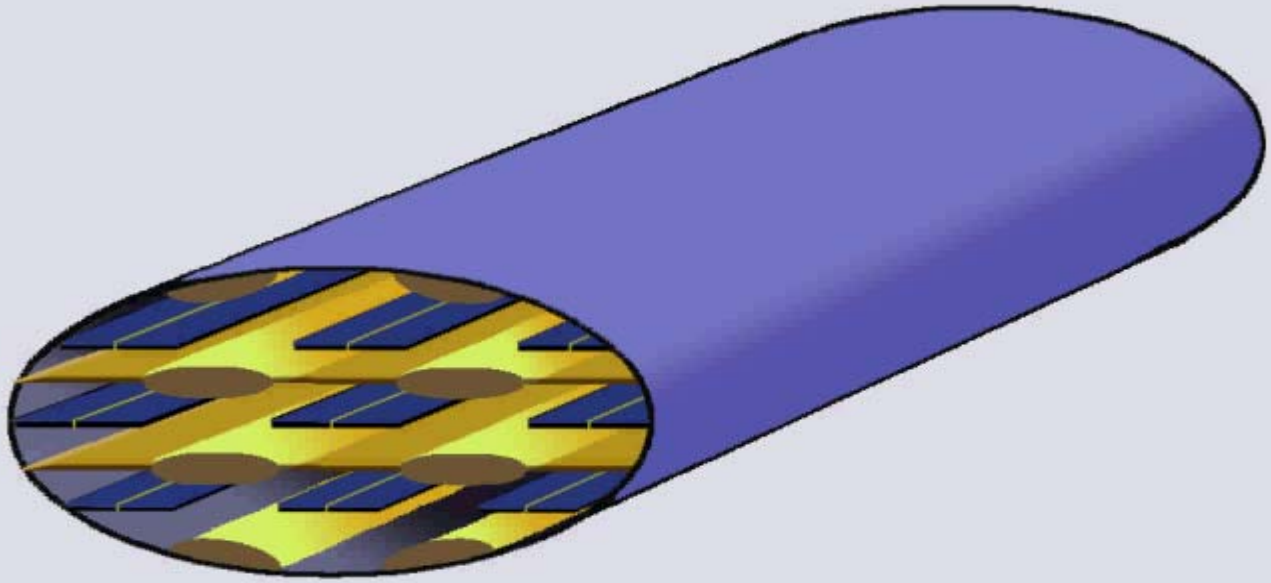
(3) Ca^{2+} makes aequorin emit photons

(4) Detect photons from cells

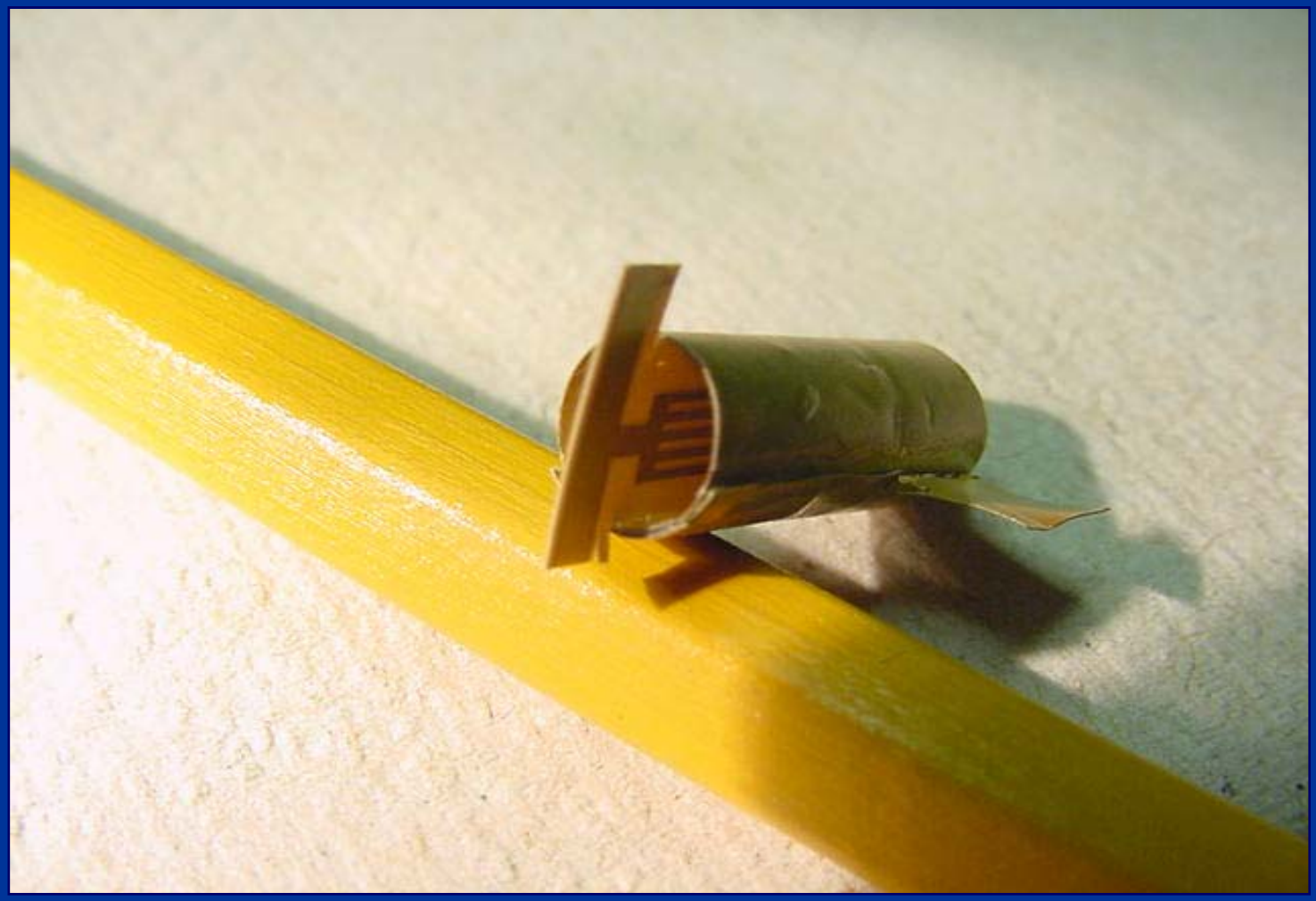
Analyte Capture



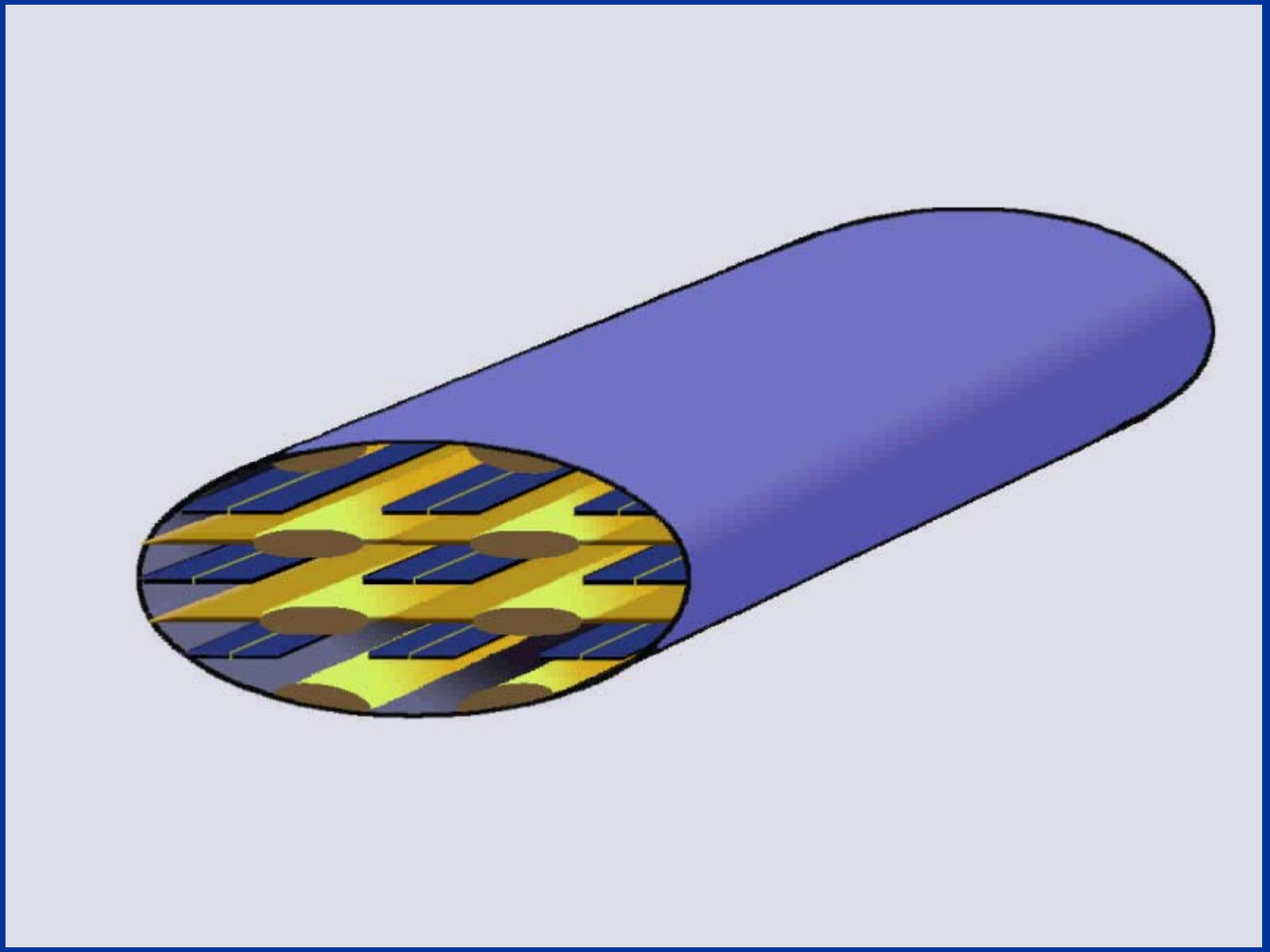
Analyte Elution



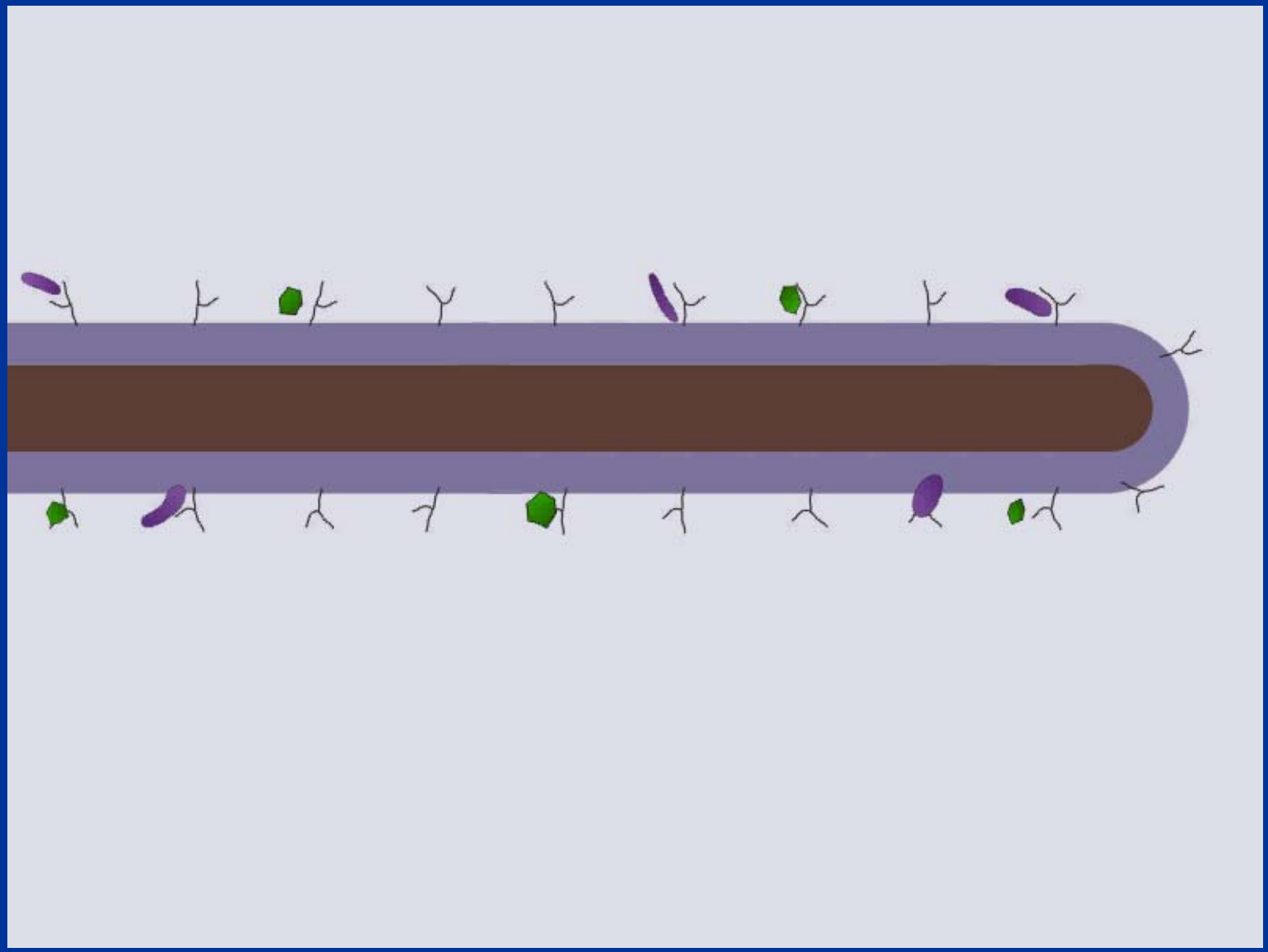
Crude Prototype Platform for Developing Capture Chemistries



Blow Up View



Analyte Elution:Chemical



Florida Department of Health
Paula Mooty and Selena Grant

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